

proteins in cell lysates. In the single molecule Co-IP technique, bait proteins in the cell lysates were pulled down onto the substrate via surface immobilized antibodies. In this study, instead of using cell lysates as a probe, single Co-IP technique is applied to single cell bait proteins. Our approach gives cell to cell variation information which was previously averaged out during lysis process. Activities of cells between different cell lines were also observed. This technique would provide detailed picture of protein interactions in cells.

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Investigation of Diffusion in Structured Samples using Fluorescence Pair Cross Correlation

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Fluorescence Fluctuation Spectroscopy methods are powerful tools for studying diffusion both in vitro and in vivo. They are, however, limited to either homogeneous samples or to fit models that rely on generalized assumptions about the heterogeneity of the environment. Actual biological samples, however, often do not fulfill these conditions. The plasma membrane, for example, shows compartmentalization that changes dynamically [1]. This high level of complexity makes evaluation of results difficult.

To better understand the effects of heterogeneities such as structures or obstacles on molecular diffusion, we monitored the diffusion of lipids in a simplified model system. We used a lipid bilayer on a nanostructured surface [2] as a simplified system to study the effect of compartmentalization on diffusion. Our samples consisted of hexagonally arranged compartments of lipid bilayers that are connected via nanometer gaps in between the metal structures. By calculating the spatial pair cross correlation functions [3] of a signal acquired while rapidly scanning over the sample, we could measure the time of diffusion of fluorescently labeled lipids between partitions. Since both diameter of the compartments and size of the gaps can be adjusted, this system allows us to study the influence of these parameters on diffusion. Furthermore, we performed Monte Carlo simulations of diffusion in similar structures. This way, we could test the effects of different interactions of the sample and the environment on the correlation functions.

[1] T. Fujiwara et al., *Journal of Cell Biology* 2002, 157, 1071.

[2] T. Lohmueller et al., *Nano Letters* 2012, 12, 1717.

[3] M. Digman et al., *Biophysical Journal* 2009, 97, 665.

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Use of Cyclic Olefin Polymer in Single Molecule Total Internal Reflection Fluorescence Microscopy

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Single molecule imaging is an established technique for many researchers in the biophysical sciences. Often, a fluorescent molecule bound near a glass or quartz surface is excited using total internal reflection fluorescence (TIRF) microscopy. TIRF excitation selectively excites fluorophores at the surface of a well or channel, thereby eliminating background fluorescence from bulk solution. However, nonspecific binding of fluorescent probes and fluorescently labeled molecules can become a limiting factor for protein association experiments. Extensive cleaning is required when using a glass or quartz substrate and the substrate is often coated with polymers such as poly-ethylene-glycol (PEG) to reduce non-specific binding. To eliminate the extensive wash and coating procedure steps we use a plastic cyclic olefin copolymer (COC) substrate that is extremely hydrophobic and can be passively activated to perform specific protein-protein interaction experiments in a very simple manner. Furthermore, COC has optical properties similar to glass making it amenable to TIRF microscopy. We first show simple processes to activate the surface in under a minute while rejecting fluorescent labeled antibody at concentrations of 1 nM. This is confirmed using objective-based TIRF microscopy and off-the-shelf COC microfluidic channels. We also show sufficient signal to noise to observe green fluorescent protein at the single molecule level using TIRF excitation. Finally, activated surfaces are used to perform a protein association experiment by detecting a sandwich of two antibodies bound to a single antigen molecule. We believe fluidic devices made of COC polymer will eliminate many of the preparation steps employed to treat other substrates allowing for easier and significantly faster protein association experiments.

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Simultaneous Position and Orientation Imaging of Polarized Fluorescence from Rod-In-Rod Semiconductor Nanoparticles on Cytoplasmic Dynein

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Sub-pixel particle tracking and polarized total internal reflection fluorescence (polTIRF) microscopy have been instrumental in understanding of motor protein function. We have developed a method combining sub-pixel tracking and polTIRF microscopy to simultaneously track position and orientation of single fluorescent particles. The sample is illuminated with circularly polarized light, and the emission is split into four polarizations and imaged with an EMCCD camera. The Yanagida lab recently published such a technique (Ohmachi et al, PNAS vol. 109, 2012). Our lab has developed a similar method, incorporating calibration routines to compensate for depolarization by phase shifts at reflecting surfaces and wavelength- and polarization-dependent detection efficiencies of the camera channels. The application of a 4x4 calibration matrix, generated by measuring deviations from theoretical polarized fluorescence intensities, compensates for depolarization caused by reflecting surfaces and channel crosstalk. Normalizing to an unpolarized sample at the relevant emission wavelength accounts for wavelength-dependent differential sensitivity of the four detection channels. Rod-in-rod CdSe core, CdS shell particles exhibit high polarization ratios; rods with dimensions of only ~5x20 nm display an average polarization ratio of 0.81. This position and orientation tracking method is currently being used to observe rotations of streptavidin-conjugated quantum rods bound to the cytoplasmic dynein AAA ring. Correlation of angular and translocation information may provide insight into mechanisms of fluctuating dynein stepping. Supported by NIH grant P01GM087253.

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Revealing Myosin's Power Stroke with High-Speed Scattering Interferometry

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How nature addresses brownian motion and achieves high processivity of molecular motors is a fundamental question in biophysics. Single molecule techniques are particularly suited to investigate nanoscopic motion with high spatial accuracy, but have struggled to provide information about structural dynamics associated with the individual motor steps. Fundamentally, the challenge has been to achieve both nanometer spatial accuracy and microsecond temporal resolution without considerably interfering with the mechanics of the step. Here, we show that interferometric scattering microscopy (iSCAT) can track the motion of both the head and stalk of myosin 5a in vitro without need of a drastic reduction of ATP concentration. We achieved 1 nm spatial precision and sub-millisecond temporal resolution using quantum dot-sized metallic nano-particles as labels. In this way, we could visualize conformational changes during the myosin 5 processive movement, a waiting state and brownian search when encountering obstacles - fundamental components of the stepping mechanism of myosin 5a. Remarkably, we observe tight control of lever-arm diffusion and motion for the duration of the step. Moreover, iSCAT signal brings additional information about z-position of the label which completes the 3D picture of myosin's moment. Our results illustrate how iSCAT can be used as a new approach to study dynamic structural changes of biomolecules in action.

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Nanoparticle Probes of Cell Surface Molecule Rotation

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Rotation of membrane proteins is a sensitive measure of their aggregation state and their interactions with other membrane species. We have used